

Factors Modulating the Secretion of Thyrotropin and other Hormones of the Thyroid Axis

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The first portion of this paper is devoted to an overview of the normal function of the hypothalamo-pituitary-thyroid axis. This section emphasizes areas of current research interest and it identifies several sites and mechanisms that are potentially important interfaces with toxins or toxic mechanisms. We then describe an *in vitro* technique for the continuous superfusion of enzymatically dispersed pituitary cells; this approach is particularly valuable in studying the dynamics of the TSH responses to the factors known (or suspected) to regulate TSH secretion *in vivo*. Using this technique, we have found that $10^{-5}M$ prostaglandin (PG) I_2 stimulates TSH secretion without altering the response to TRH ($10^{-8}M$), and that this stimulation is not due to its rapid conversion to 6-keto PGF $_{1\alpha}$. In contrast PGs of the E series (PGE $_1$ and PGE $_2$, $10^{-5}M$) increase responsiveness to TRH but have no effect alone. We found no effects of any of the other prostanoids tested (PGs A $_2$, B $_2$, F $_{1\alpha}$, F $_{2\alpha}$, thromboxanes A $_2$ and B $_2$, and the endoperoxide analog, U-44069. Somatostatin ($10^{-9}M$) inhibits TRH-induced TSH secretion, but does not alter the responsiveness to PGI $_2$. These findings suggest that somatostatin blocks TSH secretion at a point that is functionally prior to the involvement of the PGs, and perhaps does so by blocking synthesis or limiting availability of selected PGs.

I have chosen a title for this paper that reflects my perception of the twofold mission I have been given. First, I will use this initial paper of the session as a "stage setter" in that I will review the basic physiology of the thyroid axis and how its components interact under normal conditions. For the benefit of those who are not actively involved in research in this area, I will try to update the topic by pointing out areas of particular current research interest. My second goal is to emphasize, and to document by reference or presentation of data, certain aspects of the thyroid axis that are not likely to be covered by subsequent speakers. This presentation will not be laden with data concerning endocrine toxicity, but it is hoped that it will serve to identify a number of critical points in this

neuroendocrine axis at which one might predict interactions with toxins or toxic mechanisms.

Overview of the Thyroid Axis

Figure 1 presents the various elements and functional connections that comprise the neuroendocrine system of interest. Each of the three organs depicted (hypothalamus, anterior pituitary, and thyroid gland) may be considered as a target for an endogenous hormone or neurotransmitter. Of course, any of these could also be a target for some toxic compound. From the systems control standpoint, this axis operates as a classical negative feedback system with the serum concentration of thyroid hormone being the controlled variable. The immediate controller is thyroid stimulating hormone (TSH) which is secreted by the anterior pituitary. This secretory process, in turn, can be stimulated or inhibited by hypothalamic factors, and can also be inhibited by the negative feedback effects of thyroid hormones.

I have just referred to the secretions of the

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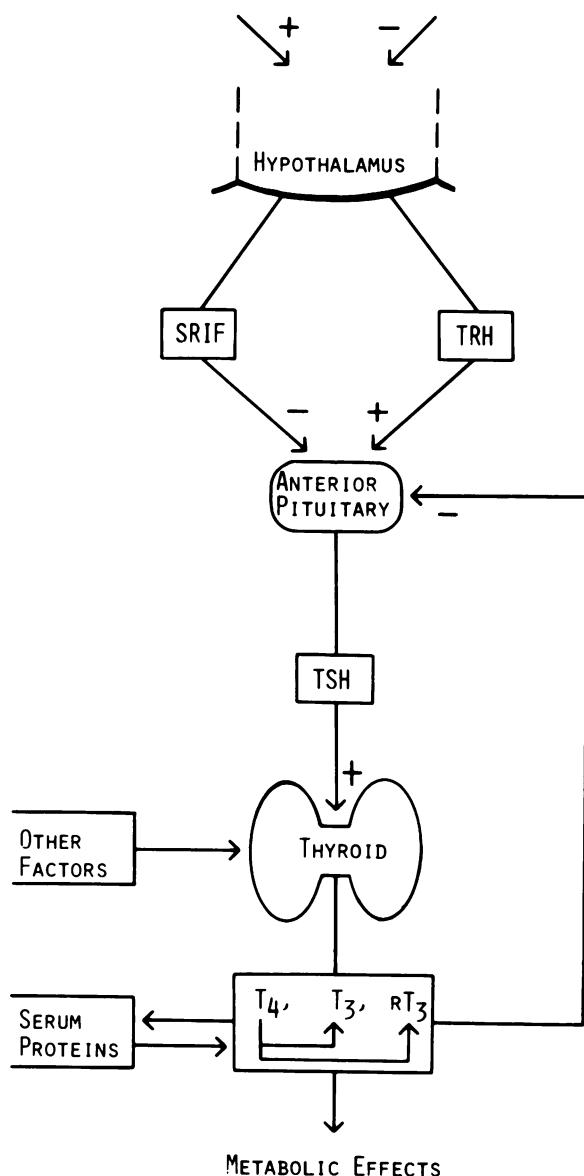


FIGURE 1. Schematic overview of the hypothalamopituitary thyroid axis.

thyroid gland simply as thyroid hormones. However, I would now like to recognize some of the individual members of this class of hormones, and briefly describe our current state of knowledge about these substances. Thyroxine (T_4) is a tetraiodothyronine secreted in relatively large quantities by the thyroid, and is, of course the classical thyroid hormone. In contrast, the other two substances listed with T_4 (T_3 and reverse T_3) are both triiodothyronines that differ structurally only in that the fourth iodine is missing from the outer ring in the case of T_3 (i.e., 3,5,3'-triiodothyronine),

whereas it is missing from the inner ring in the case of reverse T_3 (3,3',5'-triiodothyronine). In addition to this structural similarity, these two triiodothyronines are also similar in that they are derived in large part from peripheral (i.e., extrathyroidal) deiodination of circulating T_4 rather than from direct secretion by the thyroid gland. Although virtually all of the circulating T_4 is secreted directly by the thyroid only a fraction (estimates vary from 10 to 40%) of the circulating T_3 is secreted directly from the thyroid gland. Even less (if any) of the circulating reverse T_3 is secreted by the thyroid. In spite of the similarities between these triiodothyronines (structure and source), the two differ radically in one very important way—namely, their biological activity. T_3 is approximately four times as active as T_4 on a molar basis, whereas reverse T_3 is virtually inert under physiological conditions. Thus, it is clear that the peripheral deiodination of T_4 is an important source of thyroid hormones both qualitatively and quantitatively. The qualitative importance is that this mechanism can produce either the most, or the least, active thyroid hormone. From the quantitative standpoint, the most potent thyroid hormone (T_3) is derived largely from this source.

Not surprisingly, this deiodinating mechanism and the regulation of its activity has been the topic of considerable research interest recently (1). Clinical endocrinologists have particularly been interested after finding numerous circumstances in which one sees reciprocal changes in the serum concentrations of T_3 and reverse T_3 . For example, starvation or numerous severe illnesses result in an increase in circulating levels of reverse T_3 at the apparent expense of T_3 , which falls concomitantly. The enzymic processes which result in the monodeiodination of T_4 at the inner or the outer ring are known to occur in numerous tissues, primarily in the liver and kidney. However, we are just beginning to recognize some of the mechanisms that regulate these processes. I would like to point out though, that as this story has developed there has been considerable speculation that T_4 might in fact be only a prohormone (i.e., that it might have to be deiodinated to T_3 in order to be hormonally active). Although this hypothesis has attracted considerable attention and has undoubtedly been of heuristic value, it seems clear now that T_4 itself can indeed act as a hormone at least regarding some of its effects. In such cases, it may well be that the T_4 is deiodinated within the target cells in the process of exerting its effect. Nonetheless, it is the T_4 that has functioned in the classical sense of a hormone, that is, it has transmitted the message from the point of origin to the target tissue.

It is particularly germane to this symposium to point out that the monodeiodination of T_4 to either T_3 or reverse T_3 may be viewed as a branch point that has the potential to function as a very powerful peripheral regulator of the thyroid state. Consequently, this is one rather obvious place for toxicologists to direct their energies in the study of the toxicity of the thyroid axis.

Before proceeding upward in the system depicted in Figure 1, I should point out the great likelihood of the effects of toxins at another site in the periphery, namely, at the interaction between the thyroid hormones and their binding proteins in serum. Normally, these hormones are predominantly (i.e., greater than 99%) bound to these proteins. If, as most believe, only the free fraction of these hormones is active, then shifts in binding without other compensation can result in changes in the thyroid state. Many drugs are already known to alter the protein binding of thyroid hormones, and certainly any other foreign compound (or toxin) should be considered in this light as well.

Although these events located "below" the thyroid in a functional sense are incredibly important in the overall regulation of the thyroid axis, they do not detract from the importance of factors that alter secretion by the thyroid itself since this gland provides not only a portion of the active hormone pool, but it also provides all substrate for the peripheral deiodination. Of course, under normal physiological conditions, the rate of secretion by the thyroid is primarily determined by the concentration of TSH to which it is exposed. In a moment, I will return to the results of some recent experiments dealing with the factors that regulate TSH secretion. Before that, and consistent with my mission, I would like to point out that a number of factors other than TSH can also alter thyroid secretion. Recognizing that there may be overlap within the list, and with the primary mechanism (TSH), some factors worth mentioning are the following: (1) immunoglobulins—in particular, the immunoglobulin associated with Grave's disease which stimulates all aspects of thyroid hormone synthesis and secretion, probably via cyclic AMP; (2) iodine; (3) drugs—numerous antithyroid drugs act at various sites within the thyroid hormone cascade; (4) diet—of primary concern are the iodine mentioned above and the so-called "goitrogens" found in some foodstuffs; (5) adrenergic nerves—at least in animal studies, it seems clear that norepinephrine from nerve terminals within the thyroid can activate thyroid hormone secretion; (6) hormones other than TSH—for example, estrogens and glucocorticoids, which may exert their effects via binding proteins and/or TSH; and (7) age—

there are several interesting transients in thyroid hormone levels during the pre- and neonatal period, and other more gradual changes throughout life into old age. In addition to these factors which we might classify as extrathyroidal factors, we should also consider intrathyroidal factors such as cyclic nucleotides, prostaglandins, and polyamines. These are actually suspected mediators of TSH action. Unfortunately, the associated biochemistry and physiology is still loaded with uncertainty in spite of much current research activity (2). In any case, any toxicity relating to the economy of these ubiquitous substances might well be expected to affect thyroid function.

Regulation of Thyrotropin Secretion: Superfusion of Enzymatically Dispersed Pituicytes

I would now like to turn to the regulation of the secretion of the primary controller of thyroid function, i.e., TSH. This hormone is secreted by the anterior pituitary, and is under the influence of three humoral factors. The first of these is the negative feedback inhibition by thyroid hormones arriving via the systemic circulation (Fig. 1). In addition, there are two hypothalamic hormones that arrive via the hypothalamo-hypophyseal portal vessels which can alter TSH secretion; thyrotropin releasing hormone (TRH) stimulates TSH secretion, and somatostatin (SRIF) inhibits TSH secretion. I must emphasize the significance of this latter vascular connection which arises in the region of the medial basal hypothalamus as a capillary plexus, and terminates only a short distance away in the sinusoids of the anterior pituitary. Experimentally, it is often necessary to distinguish between a pituitary and a suprapituitary site of action of some substances. As you might imagine, this very closely coupled series configuration often complicates such experiments. In attempting to surmount this problem several years ago, we (3) and others (4) addressed the anterior pituitary directly by a stereotaxic approach, and still others (5) have used a more demanding approach of placing a micropipet into a single portal vessel. However, our recent efforts in this regard have been concerned with an *in vitro* system which we feel has a number of advantages over the classical static incubation of hemipituitaries.

We have established a system for the continuous superfusion of enzymatically dispersed anterior pituitary cells (Fig. 2). This system has some elements in common with those described by Lowry (6), Schrey et al. (7), and Mulder and Smelik (8). A

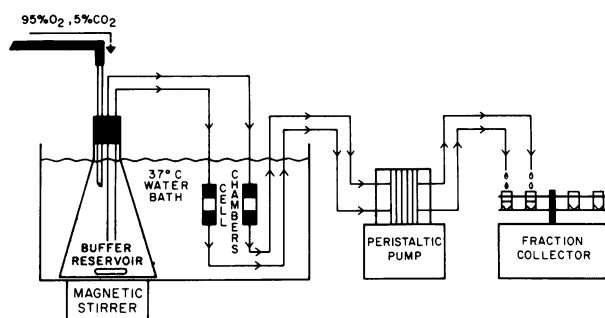


FIGURE 2. Diagram of system for continuous superfusion of enzymatically dispersed pituitary cells and collection of effluent for hormone assay.

peristaltic pump draws mixed and gassed buffer through a pair of chambers containing collagenase-dispersed pituicytes supported on Sephadex. Each column contains 6 to 10 million cells which can be exposed to various substances using valves that divert the flow just upstream from the cell columns. The effluent is collected for radioimmunoassay of TSH using a fraction collector set at a frequency appropriate for the given experiment. After a transient period of relatively high TSH release, a stable baseline is achieved and the cells are quite responsive to exogenous TRH (Fig. 3). We normally allow 2 hr for stabilization, and then we continue to use the cells for approximately 6 hr, although we have used them for as long as 24 hr on occasion.

A number of advantages of such a system over static incubation of pituitary tissue are self-evident (e.g., continuous supply of nutrients and removal of metabolites). Even if one accepts the advantages of a flowing system, we also feel that there are advantages to using dispersed cells as opposed to hemipituitaries or pituitary fragments. For exam-

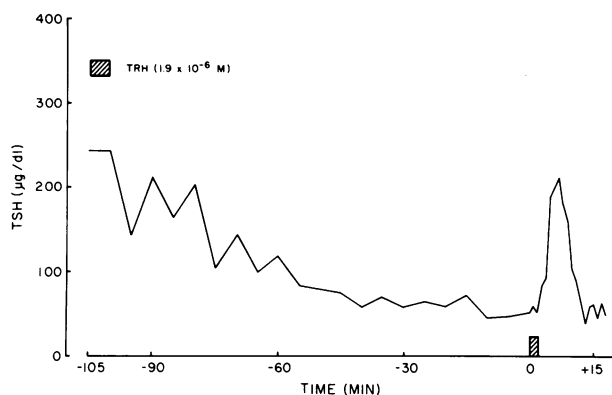


FIGURE 3. Time course of TSH release from superfused pituicytes placed on the columns at approximately -120 min. A typical response to a 2 min pulse of TRH after stabilization is also shown.

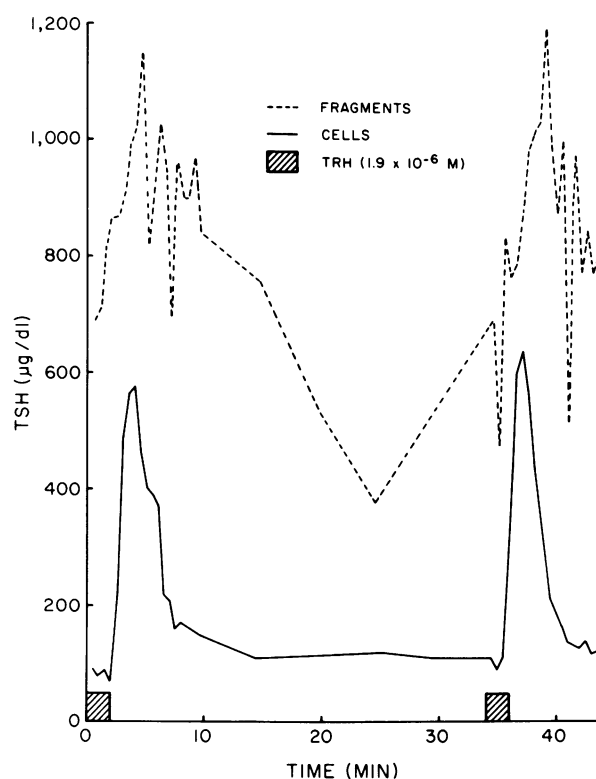


FIGURE 4. Comparison of response dynamics of dispersed cells and pituitary fragments. In the latter case, each anterior pituitary was cut into 10 pieces.

ple, if one is interested in studying the dynamics of pituitary secretion (as we are), then the dispersed cells constitute the preparation of choice, as shown in Figure 4. From these responses to 2-min pulses of TRH, it is clear that the user of dispersed cells pays a bit of a price regarding the magnitude of the response, but he gains considerably regarding stability of response.

Of course, during a typical 6-hr experiment, we want to stimulate the cells repeatedly under various experimental conditions. For this reason, it is important to know that the responsiveness of these cells is constant under constant conditions. We have provided this assurance by demonstrating consistent responses to a train of constant, nonmaximal TRH stimuli with interstimulus intervals varying between 20 and 70 min (unpublished observations). Finally, in describing the basic characteristics of this system, I should mention the effect of the second hypothalamic hormone listed on Figure 1, namely, somatostatin. As will be documented on a later slide, this peptide does not affect basal TSH secretion, but it does inhibit TRH-induced TSH secretion. It is interesting to note that even maximal doses of somatostatin fail to completely suppress TRH-induced TSH secretion.

These findings are consistent with the results from other laboratories (9, 10).

Thus the effects of somatostatin and TRH that I have described agree with the depiction in Figure 1. However, this does not address the issue of physiological roles for these two peptides in regulating TSH secretion. Perhaps the best evidence to support such roles comes from passive immunization studies from other laboratories. For example, it has been shown that the administration of TRH antiserum to conscious rats blocks the cold-induced TSH response, suggesting that endogenous TRH is involved in mediating this response (11). Similarly, the administration of somatostatin antiserum enhances both basal and TRH-stimulated TSH secretion, providing some evidence for a physiological role for this peptide as well (12).

Effects of Prostaglandins on Thyrotropin Secretion

Our primary interest in somatostatin and TRH in this superfusion system is in their usefulness as tools to help us define more clearly the roles of other substances thought to be involved in regulating TSH secretion. Recently, we have been particularly interested in using this approach to follow-up our earlier *in vivo* studies of the roles for prostaglandins (PGs) in regulating pituitary secretion (13). This earlier work concentrated on the so-called stable PGs typified by those of the E series and the F series. Since that time, numerous new members of the prostanoid family have been recognized, such as the endoperoxide intermediates (PGG_2 and PGH_2), prostacyclin (PGI_2), and the thromboxanes (14). Thus, we have recently used our *in vitro* system to investigate some of these prostanoids in relation to pituitary secretion in general; I will present some of our findings regarding TSH in particular.

One of the primary findings is that prostacyclin can stimulate TSH release on its own, but it leaves the responsiveness to TRH relatively unaffected. This can be seen in each of the three panels of Figure 5, the left of which makes use of our standard superfusion medium containing bovine serum albumin (BSA). The other two experiments were performed at a time at which we suspected that protein in the medium was inhibiting the effect of the prostacyclin. Since this was true only if the prostacyclin solutions were prepared well in advance of the experiment, we soon circumvented this problem by using only freshly prepared prostacyclin solutions. Thus, the patterns of responses seen in the three panels of Figure 5 are

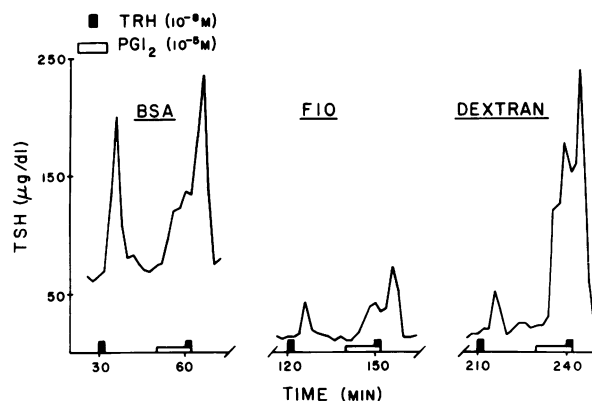


FIGURE 5. Stimulation of TSH release by prostacyclin (PGI_2) in the presence of three different types of media. Our standard medium is Hams F10 with bovine serum albumin (5 mg/ml, BSA, left panel). The medium used for the center panel was F10 without BSA, whereas the one on the right was F10 made isosmotic with dextran rather than BSA.

identical. However, it is also clear that both basal and TRH-induced TSH secretion is greatly suppressed without the normal complement of protein in the medium. Apparently this is not due to osmotic imbalance since the restoration of normal osmolality by dextran (right hand panel) does not restore normal basal or TRH-induced TSH secretion. It is interesting, but also unexplained, that the effect of prostacyclin is greater in the dextran-containing medium than in the standard BSA-containing medium. Since it is known that prostacyclin is rapidly converted to the metabolite 6-keto $\text{PGF}_{1\alpha}$, we were concerned that it might in fact be this metabolite that stimulates TSH secretion rather than the prostacyclin itself. However, we have shown that this is not the case since the same dose of 6-keto $\text{PGF}_{1\alpha}$ is completely without effect on TSH secretion (Wright and Hedge, unpublished observations).

In contrast to the effect of prostacyclin, PGE_2 accentuates the responsiveness to TRH, but does not affect basal TSH secretion (Fig. 6). The magnitude of this effect is rather small, but it is seen repeatedly and is consistent with our previous *in vivo* studies (13), and with the reports of others using static *in vitro* systems (15, 16). Although not shown here, the effect of PGE_1 is virtually identical to the effect shown in Figure 6, both qualitatively and quantitatively. The effects described for prostacyclin and PGs of the E series are quite specific in that a number of other prostanoids tested in exactly the same way have been found to be totally ineffective. Figure 7 shows typical results from experiments with PGD_2 and the stable endoperoxide analog U-44069. Negative results identical to these were also obtained upon testing

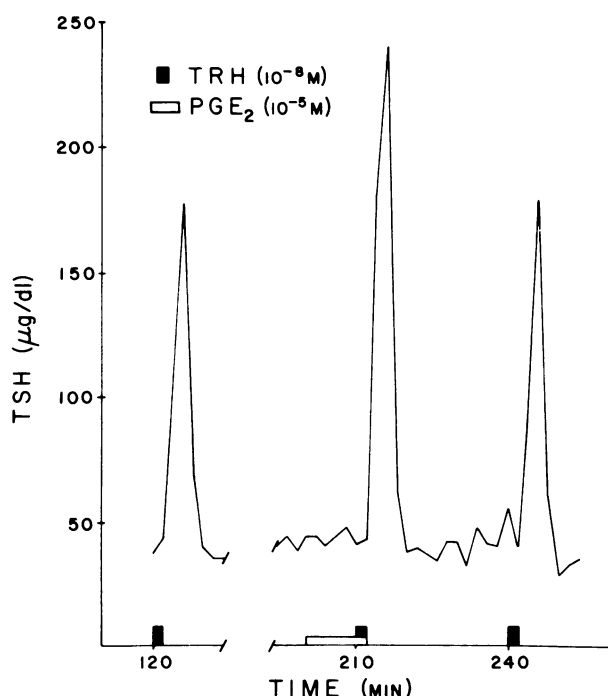


FIGURE 6. Accentuation of responsiveness to TRH by PGE_2 with normal responses of the same column shown before and after the PGE_2 treatment.

PGs A_2 , B_2 , $\text{F}_{1\alpha}$ and $\text{F}_{2\alpha}$, and thromboxanes A_2 and B_2 .

The final point to be made regarding exogenous PGs concerns the effects of somatostatin. We have seen the inhibition of TRH-induced TSH secretion by somatostatin, and we have also seen the interactions between TRH and PGs. Figure 8 presents an example of interaction among all three substances. First, I should point out that the dose of somatostatin used in this experiment exerts maximal inhibition (to approximately 35% of control) of the response to a rather high dose (10^{-7}M) of TRH. This dose of

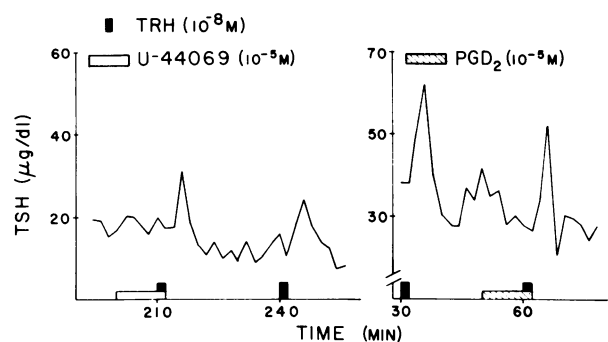


FIGURE 7. Failure of endoperoxide analog (U-44069) or PGD_2 to affect either basal or TRH-induced TSH secretion.

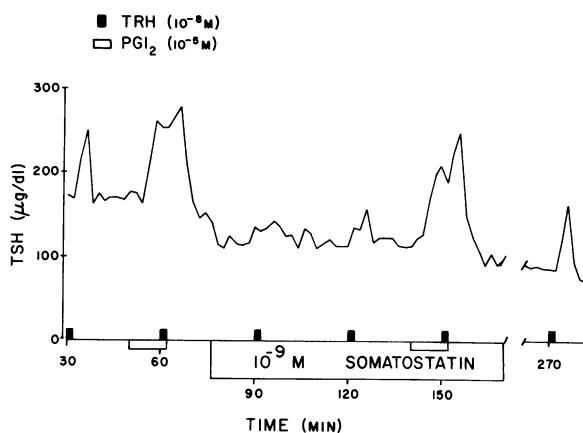


FIGURE 8. Inhibition of response to TRH, but not to prostacyclin, by somatostatin treatment, and return to normal responsiveness after stopping somatostatin.

somatostatin inhibits the response to TRH (at 10^{-8}M) as expected. However, it does not alter the effect of the prostacyclin alone. This finding, and other similar ones with PGs of the E series, suggest to us that somatostatin inhibits TRH-induced TSH secretion at some point prior to the involvement of the PGs. One possible mechanism worth considering, and worth investigating, is that somatostatin might inhibit TSH secretion by inhibiting PG formation. The PGs of the E series would seem to be particularly likely candidates to be involved in such a mechanism since they enhance responsiveness to TRH while leaving basal TSH secretion unaffected, and somatostatin inhibits TRH stimulated TSH secretion and leaves basal secretion unaffected. Although not presented in detail in this paper, we already know that the inhibition of TRH-induced TSH secretion by somatostatin can be completely reversed by replacement with exogenous PGE_1 or PGE_2 , even though these prostanoids cannot stimulate TSH secretion on their own (Fig. 8).

All of the data that I have presented thus far have been concerned with the effects of exogenous prostanoids on TSH secretion. The logical next question is to ask whether or not the endogenous pituitary PGs are actually involved in the normal regulation of TSH secretion. In extending the present studies, we have begun to examine this issue using our superfusion system. In doing so, we have made use of several pharmacological tools commonly used in PG studies. In particular, we have used indomethacin to inhibit PG synthesis and found that this greatly reduces the amount of TSH secreted in response to TRH. The fact that this inhibition can be reversed by reintroducing exogenous PGs suggests that the effect is indeed due to inhibition of PG synthesis rather than some

nonspecific effect of the drug. Conversely, we have enhanced the responsiveness to TRH by superfusing these cells with excess PG precursor (i.e., arachidonic acid). Although these studies are not yet complete, they certainly suggest that endogenous PGs are involved in regulating TSH secretion as one might predict from the findings that I have presented from our work with exogenous PGs.

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